Claims

1. A method for extracting a protein of interest from a precipitate, comprising:
   a. mixing the precipitate with a liquid in a first tank (1) to form a suspension having a first dilution factor;
   b. feeding the suspension into a first filtration unit (5) comprising a dynamic filter element adapted to produce a first retentate and a first permeate enriched with the protein of interest;
   c. diluting the suspension in the first tank (1) by adding liquid to a second dilution factor, optionally by streaming the first retentate (13) into the first tank; and
   d. recovering the first permeate enriched with the protein of interest in a second tank (7).

2. The method of claim 1 further comprising:
   e. subjecting the first permeate in the second tank (7) to a continuous concentration process in a second filtration unit comprising a cross flow filter element, thereby producing a second retentate enriched with the protein of interest and a second permeate depleted of the protein of interest;
   f. optionally diluting the suspension in the first tank (1) by streaming the second permeate to the first tank (1), thereby diluting the suspension to the second dilution factor; and
   g. either returning the second retentate enriched with the protein of interest to the second tank (7) and/or collecting the second retentate enriched with the protein of interest.

3. An industrial scaled method for extracting a protein of interest in high yield from a precipitate, comprising:
   a. mixing the precipitate with a liquid in a first tank to form a suspension having a first dilution factor;
   b. continuously feeding the suspension into a first filtration unit comprising a rotational cross flow filter element comprising a filter disc having a ceramic membrane with an average pore size between 5 nm and 5000 nm, the filter element adapted to produce a first retentate, and a first permeate enriched with the protein of interest;
   c. diluting the suspension in the first tank by adding liquid to a second dilution factor in part by streaming the first retentate into the first tank;

d. recovering the first permeate enriched with the protein of interest in a second tank; and

e. subjecting the first permeate in the second tank to a continuous concentration process in a second filtration unit comprising a cross flow filter element, thereby producing a second retentate enriched with the protein of interest and a second permeate depleted of the protein of interest;

f. optionally diluting the suspension in the first tank by continuously streaming the second permeate to the first tank, thereby diluting the suspension to the second dilution factor; and

g. either returning the second retentate enriched with the protein of interest to the second tank and/or collecting the second retentate enriched with the protein of interest.

4. The method according to claim 2 or claim 3, wherein the first permeate is continuously fed into the concentration process.

5. The method according to claim 2 or claim 3, wherein the concentration process is an ultrafiltration performed in the second filtration unit.

6. The method according to any one of the preceding claims, wherein the first permeate is collected in the second tank, and once the suspension of the first tank is completely filtrated, the first permeate from the second tank is subjected to the continuous concentration process.

7. The method according to any one of the preceding claims, wherein either the steps b) to c) or steps b) to f) are repeated until either the second dilution factor or a predetermined value of protein concentration of the suspension in the first tank has been achieved.

8. The method according to any one of the preceding claims, wherein the first dilution factor is at least 3 (1:3; parts of protein-comprising composition:total), preferably between 1 to 10, preferably between 3 to 9, preferably between 3 to 5, preferably about 3, 5, 6, 9 or 10.

9. The method according to any one of the preceding claims, wherein the predetermined second dilution factor (weight of protein-comprising precipitate to weight of total dilution) is between 6 and 70, between 10 and 70, between 20 and 50, preferably between 30 and 40, about 10, about 20, about 30, about 40, about 50, about 60, or about 70 (1:70; parts precipitate:total).

10. The method according to any one of claims 8 to 10, wherein the predetermined value of protein concentration is less than 0.1 g/L, preferably between about 0.001 to 0.1 g/L.

11. The method according to any one of the preceding claims, wherein the precipitate is an intermediate product of an alcohol fractionation process, preferably of blood plasma, more preferably of human blood plasma.

12. The method according to claim 11, wherein the intermediate product is selected from the group consisting of Cohn Fraction I (Fr I), Cohn Fraction II+III (Fr II+III), Cohn Fraction I+II+III (Fr I+II+III), or Kistler/Nitschmann Precipitate A (KN A), or a combination of KN A and one or more of Fr I, Fr II+III and Fr I+II+III.

13. The method according to any one of claims 1 to 10, wherein the precipitate is a culture supernatant or a fermentation product.

14. The method according to any one of the preceding claims, wherein the protein of interest is an immunoglobulin (Ig), preferably immunoglobulin G such as from human plasma or a recombinantly produced immunoglobulin G.

15. The method according to any one of the preceding claims, wherein the first filtration unit comprises a pressure vessel.

16. The method according to any one of the preceding claims, wherein the dynamic filter element is a dynamic cross flow filter element, preferably a rotational cross flow filter element.

17. The method according to claim 16, wherein the rotating speed of the rotational cross-filter element is between about 600 rpm and about 1200 rpm, preferably between about 800 rpm and about 1200 rpm, preferably about 800 rpm, about 1000 rpm or about 1200 rpm.

18. The method according to any one of claims 15 to 17, wherein the first filtration unit is equipped with rotating filter discs comprising ceramic membranes and optionally baffles for turbulence mixing of the content of the first filtration unit, preferably the tangential speed of the disks is between about 1 to 7 m/sec.

19. The method according to any one of the preceding claims, wherein the temperature in the first filtration unit is controlled, preferably between 2 °C and 25 °C, more preferably at about 2 °C to 10 °C.

20. The method according to any one of the preceding claims, wherein the dynamic filter element or the cross-flow filter element comprises a filtration membrane having an average pore size of between 5 nm to 5000 nm, preferably between 5 nm to 2000 nm, between 5 nm to 500 nm, between 5 nm to 200 nm, more preferably between 7 nm to 100 nm, even more preferably between 7 nm to 80 nm.

21. The method according to any one of the preceding claims, wherein the precipitate comprises a filter aid.

22. The method according to claim 21, wherein the filter aid is removed prior to the first filtration.

23. The method according to any one of the preceding claims, wherein a second tank (7) is provided to receive the first permeate (14) and/or the second retentate (16), wherein the flow velocity of the first permeate and the second permeate are controlled such that a substantially constant product volume is maintained in the second tank (7).

24. The method according to any one of the preceding claims, wherein the transmembrane pressure is from 0.1 bar to 2.5 bar, preferable from 0.2 bar to 2.4 bar, preferably from 0.4 bar to 2.0 bar, most preferably from 0.4 bar to 1.5 bar.

25. The method according to any one of the preceding claims, wherein the extraction process is further assisted by regulating the flow rate and/or the residence time of the suspension in the first filtration unit and/or the flow rate of the first retentate and/or the flow rate of the first permeate.

26. The method according to any one of the preceding claims, wherein the suspension has a pH of between about 3.0 and 9.0, preferably between about 4.0 and 7.0, between about 4.0 to 6.0, between about 4.0 to 5.0, between about 4.3 to 4.9, between about 4.4 to 4.8, more preferably about 5.0.

27. The method according to any one of the preceding claims, wherein the precipitate has a total protein concentration of about 0.5 to 6.5 % w/v, more preferably about 1.0 to 4.0 % w/v, still more preferably about 1.5 to 3.0 % w/v, most preferably about 1.8 to 2.5 % w/v, e.g. about 2.0 % w/v.

28. The method according to any one of the preceding claims, wherein the liquid comprises a buffer comprising one or more of sodium acetate, sodium phosphate, and citric acid.

29. The method according to any one of the preceding claims, wherein the precipitate is added to the first tank in the form of a suspension, pellet or preferably a paste.

30. The method according to any one of the preceding claims, wherein the product of the method is subjected to further processing including one or more of chromatography steps, virus inactivation steps, concentration and formulation such that the end product is suitable for administration to a subject.

31. A closed system (100) for extracting a protein of interest from a precipitate, comprising
    a. a first tank adapted to contain the precipitate in the form of a suspension having a first dilution factor;
    b. a first filtration unit comprising a dynamic filter element, in connection with the first tank for receiving the suspension and the filter element adapted to produce a first permeate enriched with the protein of interest and a first retentate depleted of the protein of interest, wherein the first filtration unit is adapted to return the first retentate to the first tank;
    c. a second tank in connection with the first filtration unit for recovering the first permeate enriched with the protein of interest;
    d. a second filtration unit for concentrating the first permeate in the second tank, adapted to produce a second retentate enriched with the protein of interest and a second permeate depleted in the protein of interest, wherein the second unit is adapted to return the second retentate to the second tank and/or the second permeate to the first tank.

32. The closed system (100) according to claim 31, wherein the dynamic filter element is a rotating filter element comprising one or more filter discs.

Claims

1. A method for extracting a protein of interest from a precipitate, comprising:

    a. mixing the precipitate with a liquid in a first tank to form a suspension having a first dilution factor;

    b. feeding the suspension into a first filtration unit comprising a dynamic filter element adapted to produce a first retentate and a first permeate enriched with the protein of interest;

    c. diluting the suspension in the first tank by adding liquid to a second dilution factor, optionally by streaming the first retentate into the first tank; and

    d. recovering the first permeate enriched with the protein of interest in a second tank,

wherein steps b and c are repeated until one or both of (i) a final dilution factor has been achieved in the first tank and (ii) a protein concentration of the suspension in the first tank of 0.001 g/L to 0.1 g/L has been achieved.

2. The method according to claim 1, wherein the first dilution factor is from 1 to 10.

3. The method according to claim 1, wherein steps b and c are repeated until a final dilution factor of is from 6 to 70 has been achieved in the first tank.

4. The method according to claim 1, wherein steps b and c are repeated until a protein concentration of the suspension in the first tank of 0.001 g/L to 0.1 g/L has been achieved.

5. The method according claim 1, wherein the precipitate is an intermediate product of an alcohol fractionation process of blood plasma.

6. The method according to claim 5, wherein the intermediate product is selected from the group consisting of Cohn Fraction I (Fr I), Cohn Fraction II+III (Fr II+III), Cohn Fraction

I+II+III (Fr I+II+III), Kistler/Nitschmann Precipitate A (KN A), and combinations of KN A and one or more of Fr I, Fr II+III and Fr I+II+III.

7. The method according to claim 1, wherein the precipitate is a culture supernatant or a fermentation product.

8. The method according to claim 1, wherein the protein of interest is an immunoglobulin (Ig).

9. The method according to claim 1, wherein the first filtration unit comprises a pressure vessel.

10. The method according to claim 9, wherein the first filtration unit is equipped with rotating filter discs comprising ceramic membranes and optionally baffles for turbulence mixing of the content of the first filtration unit.

11. The method according to claim 10, wherein a transmembrane pressure across the ceramic membrane is from 0.1 bar to 2.5 bar.

12. The method according to claim 1, wherein the dynamic filter element is a dynamic cross flow filter element.

13. The method according to claim 12, wherein the dynamic cross flow filter element is a rotational cross-filter element having a rotating speed of is from about 600 rpm to about 1200 rpm.

14. The method according to claim 1, wherein the temperature in the first filtration unit is controlled at 2° C. to 25° C.

15. The method according to claim 1, wherein the precipitate comprises a filter aid.

16. The method according to claim 15, wherein the filter aid is removed prior to the first filtration.

17. The method according to claim 1, wherein the extraction process is further assisted by regulating one or more of a flow rate of the suspension in the first filtration unit, a residence

time of the suspension in the first filtration unit, a flow rate of the first retentate, and a flow rate of the first permeate.

18. The method according to claim 1, wherein the suspension has a pH of from about 3.0 to 9.0.

19. The method according to claim 1, wherein the precipitate has a total protein concentration of about 0.5 to 6.5% w/v.

20. The method according to claim 1, wherein the liquid comprises a buffer comprising one or more of sodium acetate, sodium phosphate, and citric acid.

21. The method according to claim 1, wherein the precipitate is added to the first tank in the form of a suspension, pellet or paste.

22. The method according to claim 1, wherein the product of the method is subjected to further processing including one or more of chromatography steps, virus inactivation steps, concentration and formulation such that the end product is suitable for administration to a subject.

23. A method for extracting a protein of interest from a precipitate, comprising:

a. mixing the precipitate with a liquid in a first tank to form a suspension having a first dilution factor;

b. feeding the suspension into a first filtration unit comprising a dynamic filter element adapted to produce a first retentate and a first permeate enriched with the protein of interest;

c. diluting the suspension in the first tank by adding liquid to a second dilution factor, optionally by streaming the first retentate into the first tank;

d. recovering the first permeate enriched with the protein of interest in a second tank;

e. subjecting the first permeate in the second tank to a continuous concentration

process in a second filtration unit comprising a cross flow filter element, thereby producing a second retentate enriched with the protein of interest and a second permeate depleted of the protein of interest;

f. optionally diluting the suspension in the first tank by streaming the second permeate to the first tank, thereby diluting the suspension to a further dilution factor; and

g. one or both of (i) returning second retentate enriched with the protein of interest to the second tank and (ii) collecting second retentate enriched with the protein of interest;

wherein either steps b and c or steps b-f are repeated until one or both of (i) a final dilution factor has been achieved in the first tank and (ii) a protein concentration of the suspension in the first tank of 0.001 g/L to 0.1 g/L has been achieved.

24. The method according to claim 23, wherein the first permeate is continuously fed into the concentration process of step e.

25. The method according to claim 23, wherein the concentration process of step e is an ultrafiltration performed in the second filtration unit.

26. The method according to claim 23, wherein the first permeate is collected in the second tank at step d, and once the suspension of the first tank is completely filtrated, the first permeate from the second tank is subjected to the continuous concentration process of step e.

27. The method according to claim 23, wherein the dynamic filter element or the cross-flow filter element comprises a filtration membrane having an average pore size of 5 nm to 5000 nm.

28. The method according to claim 23, wherein a flow velocity of the first permeate and the second permeate are controlled such that a constant product volume is maintained in the second tank.

29. An industrial scaled method for extracting a protein of interest in high yield from a

precipitate, comprising:

a. mixing the precipitate with a liquid in a first tank to form a suspension having a first dilution factor;

b. continuously feeding the suspension into a first filtration unit comprising a rotational cross flow filter element comprising a filter disc having a ceramic membrane with an average pore size between 5 nm and 5000 nm, the filter element adapted to produce a first retentate, and a first permeate enriched with the protein of interest;

c. diluting the suspension in the first tank by adding liquid to a second dilution factor in part by streaming the first retentate into the first tank;

d. recovering the first permeate enriched with the protein of interest in a second tank;

e. subjecting the first permeate in the second tank to a continuous concentration process in a second filtration unit comprising a cross flow filter element, thereby producing a second retentate enriched with the protein of interest and a second permeate depleted of the protein of interest;

f. optionally diluting the suspension in the first tank by continuously streaming the second permeate to the first tank, thereby diluting the suspension to a further dilution factor; and

g. one or both of (i) returning second retentate enriched with the protein of interest to the second tank and (ii) collecting second retentate enriched with the protein of interest,

wherein either steps b and c or steps b-f are repeated until one or both of (i) a final dilution factor has been achieved in the first tank and (ii) a protein concentration of the suspension in the first tank of 0.001 g/L to 0.1 g/L has been achieved.